Heredity in pneumococci

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The direct control of heredity, as opposed to selective breeding to maintain races in which desired characteristics are always present, has been one of the main goals of the practical geneticist. The discovery that a specific agent—a highly polymerized desoxyribonucleic acid—can transform one strain of pneumococcus into another with different serological characteristics is, therefore, most significant. This article discusses how far the discovery can be regarded as an example of direct control of hereditary character, and explains why it should not be taken as an instance of an effect of environment on genetic make-up.

Everyday experience demonstrates that hereditary mechanisms are conservative. Offspring tend to resemble parents very closely, and it is the gross deviation from this occurrence that surprises us. This is fortunate, for, were the hereditary constitutions of individuals readily affected by environmental factors, the perpetuation of species would be gravely endangered. In spite of this conservatism, gradual evolution of species continually takes place. To understand how both conservation and gradual evolution of species occur is one of the great problems of modern biology. Even more challenging is the problem of gaining control of heredity, to guide the evolution of species to the ends which man considers good.

The science of genetics has set out to explain the mechanisms of heredity and its variation. Geneticists now understand fairly well how the characteristics of diverse species of plants and animals are conserved. Oddly enough, the research which has culminated in the formulation of the laws and theories of modern genetics did not develop through the study of the constancy of species-characteristics so much as through the study of rare mutations in these characters. These mutations, often consisting of hereditary malformations, provided essential markers for the genetic material in individual organisms, and thus enabled the geneticist to study how this genetic material is passed on to offspring. Just as mutations have been of primary importance in the study of heredity in higher plants and animals, so they are now playing an important role in the development of the genetics of bacteria. The present article deals with a remarkable kind of mutation in bacteria, that of specifically induced transformations in *Pneumococci*. However, before turning to examine this phenomenon, let us touch first upon some of the essential points of modern theories of heredity.

In probably all cells there are to be found special structures which cannot arise *de novo*, and which are exactly "copied" during cell-multiplication to form the hereditary substratum of the new cell. If any part of these structures is lost, the cell cannot by itself replace it, for it is only when these elements are themselves present that their reproduction can take place. In addition to being responsible for their own reproduction, these elements initiate reactions which lead to the formation of structures and performance of functions. The hereditary substratum is composed of units which can be differentiated both by their specific physiological activities and by their localization in cell-structures. These units, called genes, have the dimensions of large molecules, and probably differ structurally from each other at the molecular level. While one can already see in these hereditary units the makings of a system which is conservative in function, its stability would not exist without mechanisms to assure each newly produced cell a complete set of genes. Such a mechanism is known to exist in higher plants and animals, where genes are aligned in long structures called chromosomes. The number of these thread-like structures, and their size, differ for each species, and are species-characteristics. Before cell division, the genetic material in the chromosomes is duplicated, and the chromosomes split longitudinally, so that each new thread contains at least one copy of each gene. During cell-division, each daughter cell receives one of the two threads formed from each chromosome of the reproducing cell.

The greatest genetic variability is found in organisms which reproduce sexually. This is so because in the course of sexual reproduction only one-half of the chromosomal material of each parent is utilized to form the fertilized egg. Thus the new individual draws upon two different individuals for its genetic material, and is composed

only a part of the genetic patrimony of each partner. Since the new organism is not the simple sum of the total genetic material of each parent, and represents an assortment of only half of this material, new combinations of hereditary characters arise with each individual produced by a given pair of parents. At present, man cannot control which parts of the genetic material of sexually reproducing organisms will be passed on to their descendants. However, by following scientific breeding procedures, he can increase the incidence of desired properties in the offspring, or even produce true-breeding races in which the desired character is always present as long as matings are made according to strict rules.

While heredity as a whole operates to conserve the forms and functions of organisms within certain limits, new characteristics do appear from time to time. In the reproduction of every hereditary unit, or gene, there is a certain probability that the copying process will not be carried out exactly, and that a gene having new properties will appear. The probability is very small, and can be estimated only with difficulty. This spontaneous mutation of hereditary units provides a source of new kinds of hereditary material.

Since man cannot predetermine which genes are to be passed on to the offspring of sexually reproducing organisms, it has been stated that he has no control over heredity. The validity of this statement depends, of course, on what one means by control. It has been possible to create lines of plants and animals which breed true for certain desired characteristics, and it is thus clear that the hereditary substance of organisms can be manipulated for the purposes of mankind. However, one may have a very different kind of control in mind, and, indeed, this kind of control has been dreamed of by geneticists for many years. From various lines of biological research it seems likely that genes perform very specific operations in the organism. One can imagine that some day we shall be able to alter the gene specifically, to make it perform certain new desired functions. At present, there is no evidence that such control has ever been achieved. It has been possible by the use of X-rays and various other agents to increase—in a random fashion—the incidence of mutations, and this in turn has given a new degree of control over heredity. It has permitted man to increase the natural reservoir of variability inherent in genetic systems, thereby providing more new genes from which to select desirable ones. It falls short, however, of the imagined goal.

It is upon a background woven out of the efforts of geneticists to gain control over genetic systems that we should examine the nature of a rather recent discovery, viz. the transformation of pneumococcal types. At first sight, this phenomenon appears to offer the possibility of controlling the hereditary properties of an organism. It is proposed, consequently, to examine the essential aspects of the phenomenon, and then to discuss to what extent we are justified in supposing that we have at last attained the direct control of a hereditary character.

Virulent pneumococci synthesize polysaccharide capsules about themselves, which permit them to grow in their animal hosts unsuppressed by phagocytic destruction. The ability to synthesize a capsule is a constant hereditary trait. In nature, different races of pneumococci can be found, which differ from each other with respect to the chemical structure of the polysaccharide composing their capsules. The formation of a capsule of a given chemical structure is, again, constant in a given line, and therefore a hereditary trait. Distinctive kinds of antibody are formed against each type of capsular polysaccharide when the encapsulated bacteria are injected into animals. Consequently, by serological means, pneumococcal strains can be classified into groups. In a given group all races form the same kind of capsular polysaccharide, although the individual strains may differ with respect to other characters. The grouping or "typing" of pneumococci is arbitrary, but is based upon a very exact criterion.

From time to time mutation occurs in the capsule-forming mechanism, and there appear pneumococci which have a diminished power of capsule synthesis, or even a total loss of this capacity. From such mutants one may establish lines in which capsule synthesis is permanently diminished, or lines in which capsules are totally lacking. The mutated states are thus hereditary.

In 1928, Griffith discovered that unencapsulated pneumococci could be made to form capsules again, if they were injected into mice simultaneously with heat-killed encapsulated pneumococci. This discovery was particularly remarkable, since it was evident from Griffith's experiments that the induced capsule was identical with that formed by the heat-killed bacteria, and independent of the origin of the unencapsulated pneumococci in which the induction had occurred. Thus, an unencapsulated bacterium which had been derived from a Type II encapsulated race could be made to synthesize the capsule of a

Type III race, provided the induction was done in the presence of heat-killed Type III bacteria. From this basic observation the phenomenon has received its name of 'transformation of pneumococcal types.'

It seemed likely that this transformation was due to the presence of a specific agent in encapsulated bacteria. Study of the phenomenon was taken up in the laboratory of Avery, where it was found that the induction could be done with cell-free extracts of encapsulated pneumococci, acting *in vitro* upon cultures of unencapsulated pneumococci growing in a special medium. After eleven years of study of the inducing agent, Avery and his co-workers MacLeod and McCarty published the results of their research upon its chemical nature (1944). All chemical and biochemical evidence indicated that the transforming agent (TP) was probably a highly polymerized desoxyribonucleic acid. The publication of this result has roused much comment from both geneticists and chemists. From Griffith's experiments, geneticists had become interested in the transformation phenomenon, since it seemed that for the first time man was inducing a specific inheritable change in an organism. That the inducing substance should be a nucleic acid of the desoxyribose type excited still greater interest, for this is a substance which in higher plants and animals is known to occur only in the chromosomes. For many years, geneticists have speculated about the chemical nature of genes, and because proteins were known to have special structures and biological activities, it was supposed that the protein parts of the chromosomes were differentiated to give rise to gene-specificity. Before the studies on the pneumococcal TP were published, there had been no reason to believe that nucleic acid molecules in the chromosomes had a role in determining the specific properties of each gene. Chemists and biochemists were similarly interested by the studies on the TP of pneumococcus, since it had been supposed, on the basis of admittedly limited chemical evidence, that the desoxyribonucleates isolated from such different sources as wheat germ and calf thymus were chemically identical. If the transforming activity of the desoxyribonucleic acid extracts of encapsulated pneumococci is due entirely to their content of this substance, it follows necessarily that nucleic acids of the desoxyribose type must have diverse biological specificities, and hence must also have diverse chemical structures. This is evident from the experiments of McCarty and Avery (1946*a*), who isolated in purified form the desoxyribonucleates of three different types of encapsulated pneumococci. Each preparation induced unencapsulated pneumococci to form capsules, but each transformed the bacteria into a different type. The type of polysaccharide found in the induced capsule depended always upon the source of the transforming extract. Thus the kind of new synthetic activity acquired by the transformed bacteria was determined by the origin of the nucleic acid with which the induction was accomplished. It should be noted also that desoxyribonucleic acid of calf thymus is totally inactive as an inducing agent.

It is difficult to be certain that no other material in the desoxyribonucleic acid extracts contributes to their specific activity, for these extracts are active in trace amounts. However, extensive serological, chemical, and biochemical studies carried out by McCarty and Avery (1946), and by Hotchkiss (1948), indicate that no complex organic sustance other than desoxyribonucleic acid is detectable in these extracts. Furthermore, biological activity is destroyed by any agent which depolymerizes the nucleic acid. Thus, transforming activity is rapidly destroyed by crystalline desoxyribonuclease, a specific depolymerase, but is untouched by proteolytic enzymes or ribonuclease. Apparently, therefore, the polymerized desoxyribonucleic acid is solely responsible for the biological activity of the pneumococcal extracts.

As a biological agent, the capsular TP of pneumococcus presents some interesting features, since it has a dual action in the transformed bacterium. It induces both capsule formation and its own reproduction by the bacterium. Thus, once transformed, the bacterium passes a copy of the TP on to its progeny, and they, too, form capsules. The TP therefore possesses properties attributed to genes, in that genes also have this same dual role. We may, therefore, suppose that the capsular TP is simply a part of the genetic substratum of the encapsulated pneumococcus, which has been extracted in active form and introduced into the unencapsulated bacterium. Were this the case, we should expect to find other resemblances between the capsular TP and genes and, as well, other gene-like agents in the desoxyribonucleic acid extracts.

Inside the bacterium, the capsular TP undergoes spontaneous mutations upon very rare occasions, much as genes do (MacLeod and Krauss 1947; Taylor, 1949). These mutations give ri

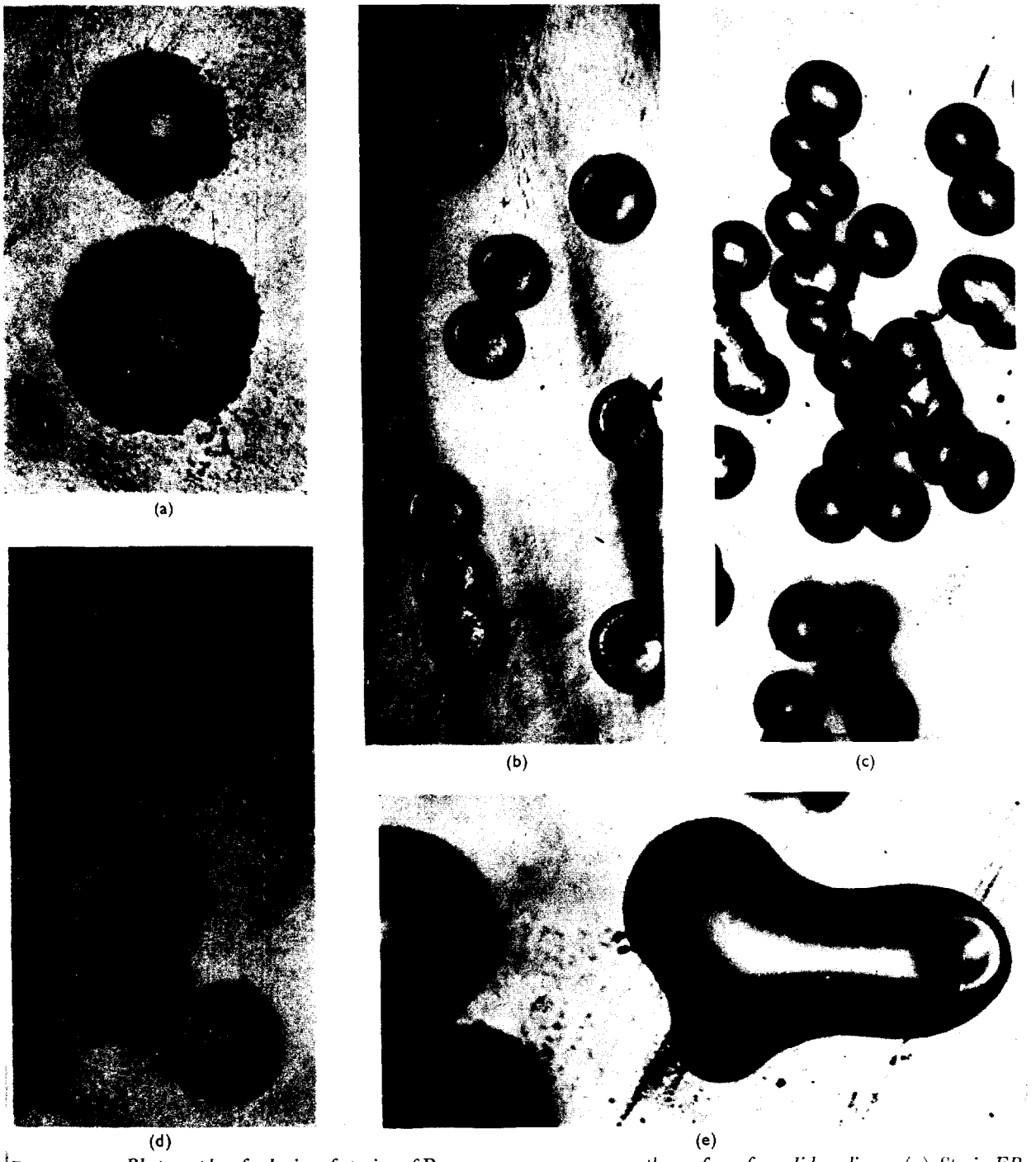

FIGURE 1 – *Photographs of colonies of strains of* Pneumococcus *grown on the surface of a solid medium.* (a) *Strain ER, an extremely rough colony. The bacteria are not encapsulated, and form long chains which become matted.* (b) *Strain R, rough colony. These also are unencapsulated, but do not form long chains.* (c) *Strain SIII–1, intermediate between rough and smooth. These bacteria form small amounts of Type III capsular polysaccharide. Reduced capsule formation is due to mutation of the capsular transforming agent.* (d) *Strain SIII–2, smooth colony. These bacteria are encapsulated, but also form less polysaccharide than does the normal SIII–N strain, owing to mutation of the capsular transforming agent in this race.* (e) *Strain SIII–N, smooth colony. Encapsulated with Type III polysaccharide.* (× 25)

to capsular transforming agents having modified biological activities (see figure 1). The mutated agents can be introduced into unencapsulated pneumococci by the transformation technique, where they induce the formation of modified capsules (see figure 2). The transformed pneumococci

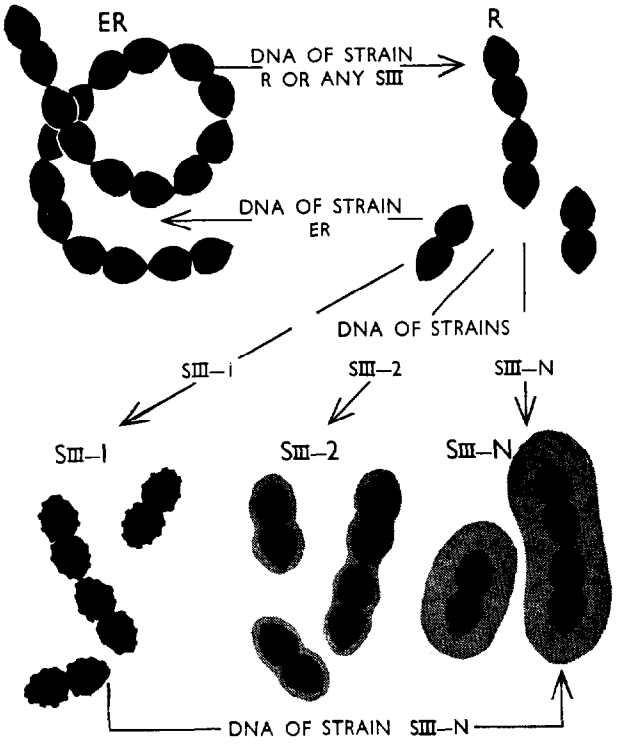

FIGURE 2 – *Diagram of some of the transformations which can be induced in the strains shown in figure 1. DNA = desoxyribonucleic acid fraction of the indicated strain. Red = Type III capsular polysaccharide. Note that the DNA fractions of the SIII strains have two different specific transforming activities: ER to R, and R to the SIII condition which corresponds to the source of DNA.*

copy the mutated agent faithfully and pass copies on to their progeny, just as occurs in the transformations with an unmutated capsular TP.

It has been found also that a given desoxyribonucleic acid extract has more than one kind of transforming activity. The desoxyribonucleate of Type III pneumococci, for example, contains at least two known kinds of transforming agents. One induces capsular polysaccharide formation in unencapsulated strains, and a second induces a distinctive morphological change in a particular strain of pneumococci (see figure 2).

In summary, it seems probable that the specific transforming agents are composed of desoxyribonucleic acids having different specific activities, and that they are parts of the genetic substrata of the bacteria from which they are isolated. In specific transformations, these agents become incorporated into the genetic constitution of the bacteria transformed, endowing them with both new genetic elements and new hereditary characters.

To what measure have these studies advanced our control of genetic systems? One should not conclude that we have finally acquired control over the biological properties of a genetic substance, for we have not yet influenced the properties of the gene-like transforming agents themselves, nor have we created a genetic element *de novo*. In pneumococcal transformations, a ready-made agent is taken from one bacterium and made to interact effectively with another, thereby endowing the transformed bacterium with new hereditary properties. Consequently, in one respect, these transformations resemble hybridizations, in that they are the manipulation of preformed genetic material. We may hope some day to be able to modify at will the properties of transforming agents, for these agents are easily accessible outside the cell and in solution in a test-tube. Perhaps, even, chemists will be able to explore the chemical structures of these agents sufficiently to permit the synthesis of gene-like agents from relatively simple organic molecules.

While there is every reason to believe that through a study of these transformations we shall gain much new insight into the structure and function of genetic determinants as a whole, there is, at present, no basis for supposing that the discovery of the transformation phenomenon represents a clean break with the concepts of modern genetics. Although these transformations are induced by agents which are introduced into the environment of growing bacteria, and can be called 'environmental agents,' the origin and properties of the transforming agents are such as to make one believe that we are concerned here with the introduction of new genetic material into the bacterium, and not with the modification by environmental factors of the hereditary substratum which the bacterium already possessed. The transforming agents which we isolate from various bacterial strains have their own unique properties, and for the moment their properties are as uncontrollable by man as those of the gene.

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